Nonthermal Inactivation of *E. coli* in Fruit Juices Using Radio Frequency Electric Fields

David J. Geveke[1], Christopher Brunkhorst[2], Peter Cooke[1], and Xuetong Fan[1]

[1]Food Safety Intervention Technologies Research Unit, Eastern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, 600 East Mermaid Lane, Wyndmoor, PA 19038
[2]Princeton University, Princeton Plasma Physics Laboratory, Princeton, NJ 08543

Radio frequency electric fields (RFEF) processing to inactivate bacteria in apple juice at moderately low temperatures has recently been developed. The process is similar to the pulsed electric fields process, except that the power supply is continuous rather than pulsed; therefore, the capital costs may be less. Orange juice and apple cider containing *Escherichia coli* K12 were exposed to electric field strengths of up to 25 kV/cm at frequencies ranging from 21 to 40 kHz. Following treatment at an outlet temperature of 65°C, the population of *E. coli* K12 in orange juice was reduced by 3.4 log relative to the control. Increasing the electric field strength and temperature and decreasing the frequency enhanced the inactivation. The electrical cost of the RFEF processing was approximately $0.0017 per liter of orange juice. There was no change in brownness of orange juice nor was there any loss of ascorbic acid as a result of RFEF treatment. The population of *E. coli* K12 in apple cider was reduced by 4.8 log following RFEF processing at 60°C, whereas, thermal processing at the same time and temperature had no effect. Increasing the electric field strength and temperature enhanced the inactivation; however, there was no

enhancement at lower frequency. Scanning electron microscope images of *E. coli* K12 that were thermally processed and RFEF processed indicated that the mechanisms of thermal and RFEF inactivation were dissimilar. The results of the present study provide the first evidence that the RFEF process inactivates bacteria in orange juice and apple cider containing solids at moderately low temperatures.

Introduction

Outbreaks of food-borne illness caused by contaminated beverages such as orange juice and apple cider still occur despite increased efforts to improve preharvest intervention. Meanwhile, consumers are demanding that these products retain maximum freshness. Hence, nonthermal pasteurization processes are actively being developed. High hydrostatic pressure and ultraviolet light processing have been commercialized to a small extent, but they each have problems which limit their scope. High hydrostatic pressure processing is a batch operation and is much more costly than traditional heat pasteurization. Ultraviolet light processing of opaque juices requires that the juice be formed into a thin film. This restricts the flow rate and the commercial applications (*1*).

High electric field processing has the potential to be commercially adopted on a large scale because it does not suffer from the above problems. It is a continuous operation that is scalable to commercial flow rates. Radio frequency electric fields (RFEF) processing inactivates microorganisms in liquids at low temperatures (*2*). A simple schematic of the process is shown in Figure 1. In this case, a 20 kV/cm electric field strength is produced by separating two parallel plate electrodes by 1 cm and applying a peak voltage of 20 kV to the electrodes. Various other combinations of separation distance and voltage may be used as long as the field generally remains above *5* kV/cm (*3*, *4*). So, for instance, the electrodes could be spaced farther apart in order to accommodate a higher flow rate provided that the voltage increased correspondingly. Other electrode geometries besides parallel flat plates are also possible (*2*, *5*). The voltage can be applied by several different means. In pulsed electric field (PEF) processing, a charging power supply produces a high voltage and a high speed electrical switch delivers the stored energy to the electrodes. The power supply must then be recharged which results in pulsed processing. Bipolar waveforms as presented in Figure 2 are extensively used in PEF processing. In RFEF processing, an AC power supply continuously provides the high voltage as illustrated in Figure 3.

This potentially simpler method of generating high electric fields may have lower capital and operating costs than those associated with PEF processing.

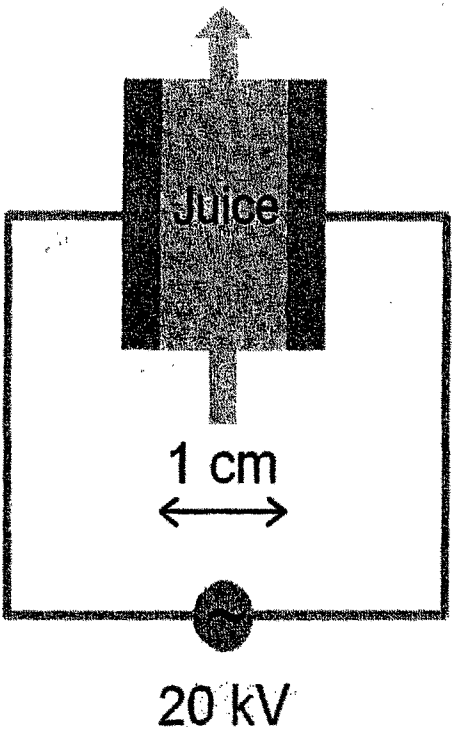

Figure 1. Schematic of RFEF process. Juice flows between two parallel plate electrodes separated by 1 cm with a 20 kV alternating current across them.

Nonthermal inactivation of microorganisms is thought to occur by electroporation (*6*). In an electric field, a voltage is formed across the cell membrane. The opposite charges on either side of the membrane are attracted to each other and the membrane becomes thinner. At a sufficiently high voltage, pores are formed in the membrane and the cell ruptures (*7*).

Nonthermal RFEF processing using bench scale equipment has been shown to be effective at inactivating *Saccharomyces cerevisiae* (*2*) and *Escherichia coli* K12, hereafter referred to as *E. coli* (*8*). Recently, a pilot plant scale RFEF processing system has been designed, fabricated, and assembled (*9*). RFEF processing reduced the population of *E. coli* in apple juice by 2.7 log at 60°C and a hold time of 3 s, whereas conventional heating at the same conditions

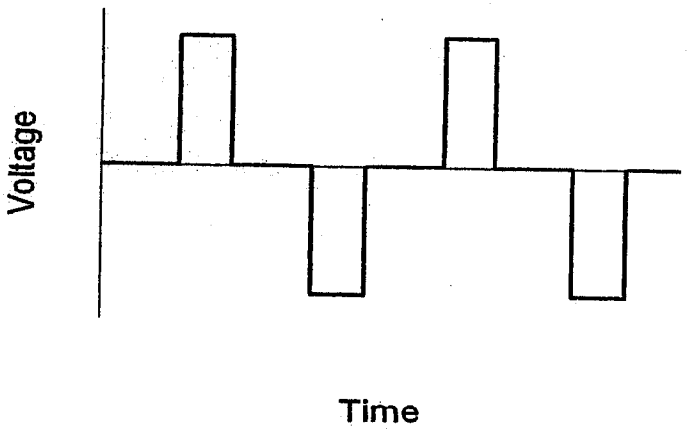

Figure 2. Example of bipolar pulses used in PEF processing.

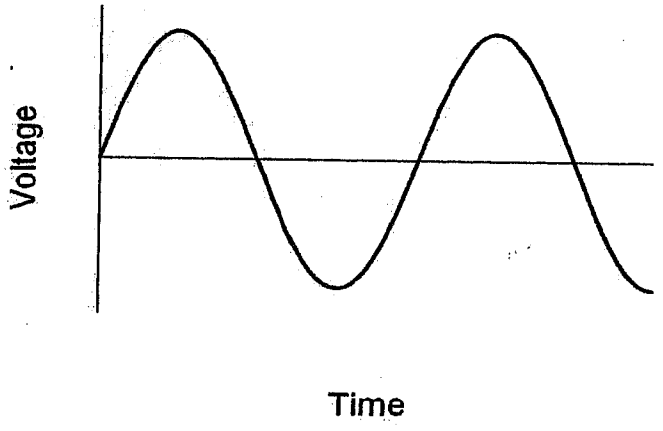

Figure 3. Example of sinusoidal waveform used in RFEF processing.

had no effect. The remainder of this chapter will cover RFEF equipment, additional inactivation results, nutritional and quality results, RFEF modeling, scanning electron microscopy results, costs, and the outlook for the future.

Radio Frequency Electric Fields Equipment

Recently, Geveke and Brunkhorst have developed a pilot plant RFEF process (*9*). The power supply that was constructed consisted of an 80 kW RF power source (Ameritherm, Scottsville, NY, model L-80) and a custom designed matching network (Ameritherm) that enabled the RF energy to be applied to a resistive load over a frequency range of 21.1 to 40.1 kHz (Figure 4). The supplied voltage and current were measured using a voltage divider (Ross Engineering, model VD15-8.3-A-KB-A), current probes (Pearson Electronics, CA, model 411) and an oscilloscope (Tektronix, model TDS224).

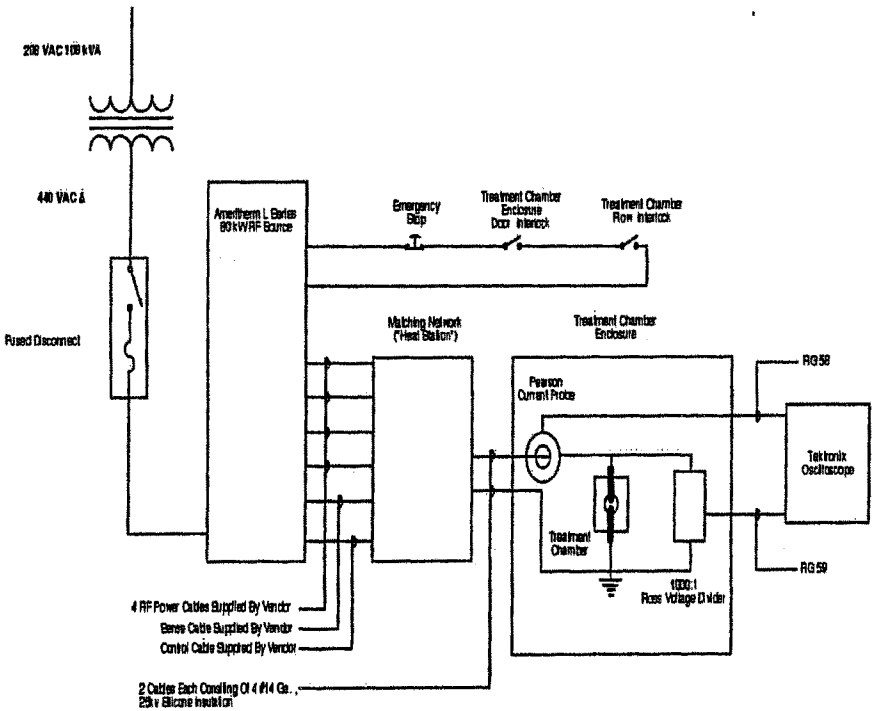

Figure 4. Electrical diagram of 80 kW RFEF system.

A novel treatment chamber was designed and fabricated to apply high electric fields to the juices (*8*). The treatment chamber was constructed of Rexolite, a transparent cross-linked polystyrene copolymer (C-Lec Plastics, Philadelphia, PA). It was designed to converge the liquid into a narrow flow area in order to reduce the power requirement (*10, 11*). Liquid entered and exited the Rexolite chamber through the annuli of cylindrical stainless steel electrodes (Swagelok, Solon, OH, part no. SS-400-1-OR) as shown in Figure 5. The electrodes were separated by a thin partition with a channel of circular cross section through the center. The diameter and length of the channel were 1.2 mm and 2.0 mm, respectively, for the experiments done on orange juice. A 9.0 mm space between the end of each of the electrodes and the central channel prevented arcing. For the apple cider experiments, the diameter and length of the central channel were scaled up to 1.4 mm and 2.3 mm, respectively, in order to achieve higher flow rates. It was determined that the space between the electrodes and the channel could be reduced, so as to maximize the electric field, to 2.0 mm without encountering arcing. The output of the RFEF power supply was connected to the electrodes such that the electric flux lines were approximately perpendicular to the direction of the liquid flow.

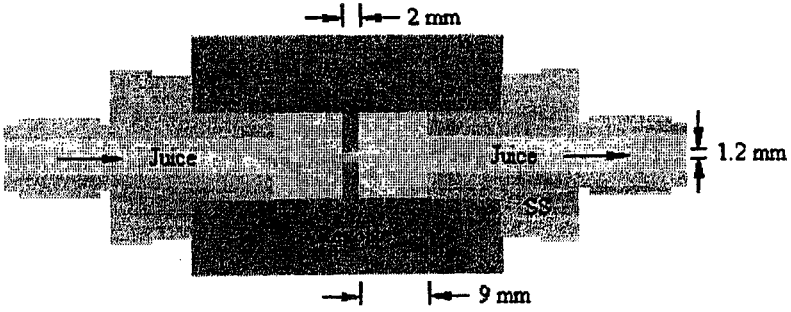

Figure 5. Cross-section of converged co-field treatment chamber, used in orange juice experiments, including Rexolite insulation and two stainless steel electrodes. The diameter and length of the central channel are 1.2 mm and 2.0 mm, respectively, and the space between the end of each of the electrodes and the central channel is 9.0 mm.

The treatment chambers can be connected to the RFEF power supply in several different ways. One configuration, that was used for the apple cider experiments, has the cider flowing in series through one or more treatment chambers as shown in Figure 6. The first electrode on each of the treatment chambers is grounded. The remaining electrode on each of the treatment chambers is connected to the RFEF power supply in parallel. Upon exiting the treatment chamber the cider flows through a 1.8 m section of plastic tubing having an internal diameter of 3.2 mm. The purpose of this plastic tubing is to electrically isolate the treatment chamber from the surrounding equipment and ensure that the maximum field is achieved within the chamber. The temperature of the cider rises during RFEF processing due to ohmic (resistance) heating. Therefore, the juice flows through heat exchangers after each treatment to control the processing temperature. Another way of connecting the treatment chambers to the power supply, that was used in the orange juice experiments, is presented in Figure 7. Two chambers are joined by stainless steel tubing. The inner electrodes between the chambers are connected to the RFEF power supply. The outer electrodes are grounded. The advantage of this setup is that there is no concern about isolating the chambers from the surroundings. The disadvantage is that, for a given field, the temperature rise is twice that for a single treatment chamber.

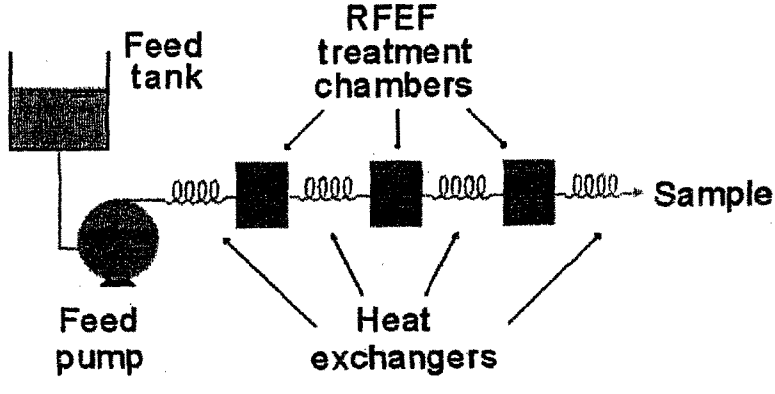

Figure 6. Schematic diagram of a continuous RFEF process, used in apple cider experiments, including three treatment chambers in series with intercooling.

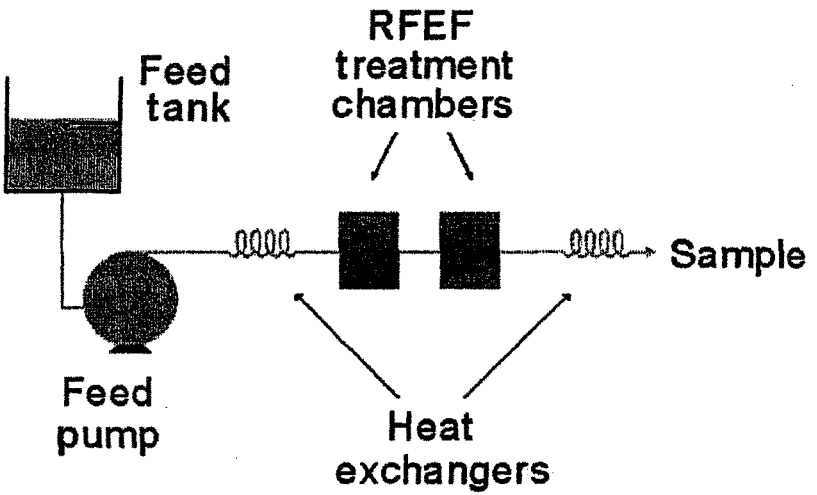

Figure 7. Schematic diagram of a continuous RFEF process, used in orange juice experiments, including two treatment chambers in series without intercooling.

The experimental system included a stainless steel feed tank and a progressing cavity pump (Moyno, Springfield, OH; model 2FG3) that supplied the juice to the RFEF treatment chambers at a flow rate ranging of 1.4 to 1.5 l/min. Multiple treatment chambers and turbulent flow within the treatment chambers improved the processing uniformity. The juice was exposed to intense RFEF in each chamber for 110 to 190 μs. At a frequency of 21.1 kHz, the liquid was exposed to at least one complete AC cycle in each chamber. A back pressure of 1 atmosphere gauge minimized arcing. A 0.24 m^2 stainless-steel heat exchanger (Madden Manufacturing, Elkhart, IN; model SC0004) and a temperature controller (Cole-Parmer, model CALL 9400) were used to regulate the inlet temperature to the initial treatment chamber and to intercool the juice between chambers as shown in Figures 6 and 7. The time for the liquid to travel from the chambers to the intercoolers ranged from 1.5 to 2 s. The temperatures of the juice immediately before and after the chambers were measured with 3.2 mm diameter chrome-constantan thermocouples (Omega Engineering, Inc., Stamford, CT). The temperatures were continuously logged to a data acquisition system (Dasytec USA, Amherst, NH, Dasylab version 5.0). The juice was

quickly cooled after exiting the last chamber to less than 25°C using a stainless-steel heat exchanger (Madden Manufacturing, model SC0004). The time for the liquid to travel from the treatment chamber to the sample cooler ranged from 1.5 to 2 s.

Controls were performed to determine the effect of temperature alone. In order to ensure that the control liquid received the same time and temperature history as the treated liquid, the converged treatment chambers were replaced with ohmic heating chambers. These chambers consisted of stainless steel electrodes (Swagelok, Solon, OH, part no. SS-400-1-OR) inserted into 102 mm lengths of 6.4 mm ID plastic tubing. The ohmic heating chambers quickly brought the juice temperature up to the desired temperature. The control juice was identically held for 1.5 to 2 s before cooling.

Modeling of Radio Frequency Electric Fields

The anisotropic electric field strengths within the treatment chamber can be modeled with finite element analysis software such as QuickField™ (Tera Analysis Ltd, Svendborg, Denmark, version 5.0). Figure 8 presents the model's results for an electric field strength of 20 kV/cm within the converged section of the treatment chamber shown in Figure 5. The liquid flows through the electrode and enters a field-free region. It then flows into the central channel where the field is quickly raised to 20 kV/cm. The field within the channel is nearly uniform which ensures that all of the liquid is treated equally. The uniformity improves the energy efficiency of the process. By minimizing the regions within the treatment chamber where the electric field is too low to inactivate bacteria and only heats the liquid, approximately less than 5 kV/cm, the energy loss is minimized. Similarly, by minimizing the regions where the field is higher than needed to inactivate bacteria, the energy loss is minimized. Thus, the outlet temperature is lessened and the liquid is not overly treated.

RFEF Nonthermal Inactivation of *E. Coli* in Orange Juice

The recently developed 80 kW RFEF pilot plant system successfully inactivated *Escherichia coli* K12 in pulp free orange juice at nonthermal conditions. The extent of microbial inactivation is dependent on the electric field strength, frequency and temperature.

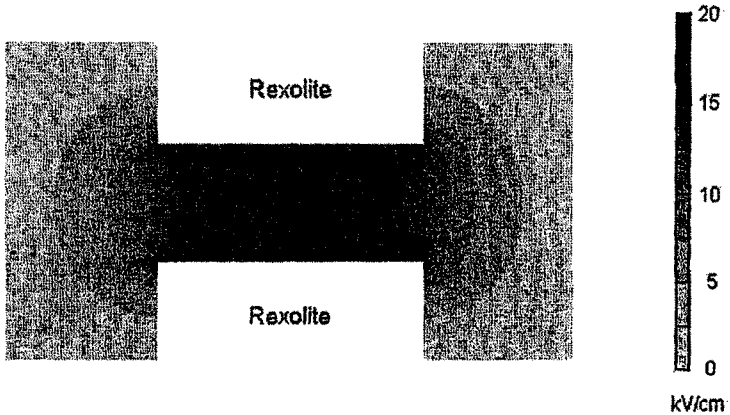

Figure 8. Modeled anisotropic RFEF strength within the converged section of the treatment chamber shown in Figure 5.

A series of experiments were performed at 21.1 kHz to determine the effects of electric field strength and temperature on inactivation. The RFEF process with two treatment chambers in series was used as shown in Figure 7. The treatment chambers used were the same as presented in Figure 5. The flow rate of orange juice was 1.4 l/min. The population of *E. coli* in orange juice was reduced by 3.2 log after being exposed to a 15 kV/cm peak electric field at a treatment time of 190 μs, inlet temperature of 40°C, outlet temperature of 65°C, and hold time of 2 s (Figure 9). Increasing the field strength to 20 kV/cm at the same temperature resulted in a reduction in *E. coli* of 3.9 log. When the juice was ohmicly heated at the same frequency, 21.1 kHz, to the same outlet temperature, 65°C, and held for the same time, 2 s, the population of *E. coli* was reduced by only 0.5 log. Therefore, RFEF processing reduced the population of *E. coli* in orange juice by 3.4 log relative to the control. The nonthermal inactivation is believed to be due to dielectric breakdown of the cells (*12*). Using the same RFEF pilot plant system, *E. coli* in apple juice was reduced by 2.1 log after being exposed to a 20 kV/cm peak electric field at a treatment time of 190 μs, outlet temperature of 65°C, and hold time of 2 s (*9*). The results of the present study successfully extended the RFEF process to inactivating *E. coli* in orange juice.

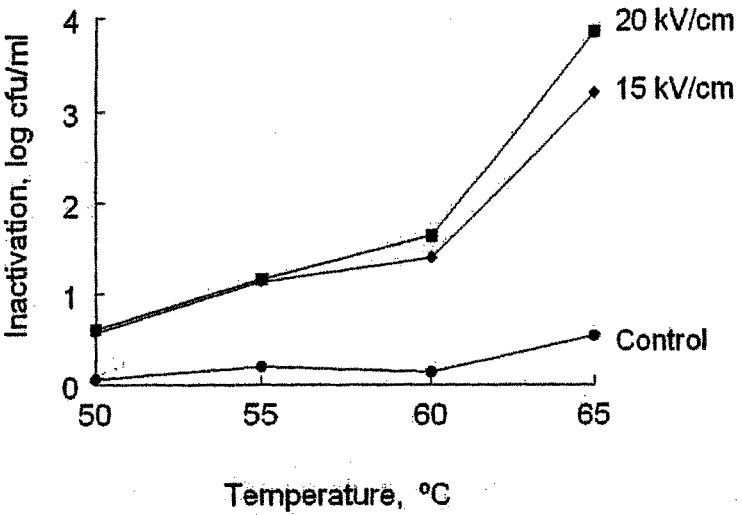

Figure 9. Effects of temperature and electric field strength on the inactivation of E. coli at 190 μs RFEF treatment time and 2 s hold time (1.4 l/min flow rate). Means of two replicate experiments.

Experiments were conducted to determine the effect of frequency on inactivation. The inactivation of *E. coli* in orange juice was substantially increased as the frequency was decreased from 40.1 kHz to 21.1 kHz as shown in Figure 10. Similar results were obtained in previous studies. Using a bench scale RFEF system, a significantly greater inactivation of *E. coli* in apple juice occurred at frequencies of 15 and 20 kHz compared to frequencies of 30 to 70 kHz (*8*). Using a pilot plant system, greater inactivation of *E. coli* in apple juice was observed as the frequency was decreased from 40.1 kHz to 21.1 kHz (*9*). These results are extremely interesting, not only because they indicate that the RFEF process could be more efficient at even lower frequencies, but also because RFEF equipment costs should be significantly less at lower frequencies as well.

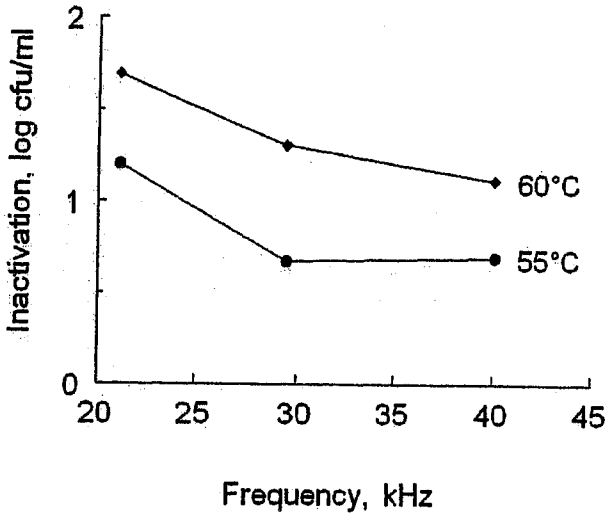

Figure 10. Effect of frequency on the inactivation of E. coli at 20 kV/cm, 190 μs RFEF treatment time and 2 s hold time. Means of two replicate experiments.

Electrical Costs of RFEF Processing of Orange Juice

The energy costs of alternative pasteurization processes are an important factor in determining whether the new technologies will be commercialized. The electrical costs were estimated for the case of RFEF processing of orange juice at 15 kV/cm and 65°C. At these conditions, the population of *E. coli* was reduced by 3.2 log and the energy applied was approximately 120 J/ml. The estimated energy required for a 5 log reduction using pulsed electric fields (PEF) ranges from 100-400 J/ml (*13, 14*). It is probable that the RFEF electrical costs for a 5 log reduction will be similar to those of PEF as they are both considered electroporation processes (*8*). Based on the U.S. Department of Energy's data for the average industrial electric price for the year 2004 of $0.051/kWh, the energy cost for the RFEF process was approximately $0.0017 per liter of orange juice. For comparison, conventional thermal pasteurization, with heat regeneration or recovery, costs only $0.0005 per liter.

Nutrition and Quality of RFEF Processed Orange Juice

Two of the commonly occurring degradations in juice quality are non-enzymatic browning and loss of ascorbic acid. An experiment was conducted to ascertain the effect of RFEF processing on these two aspects of juice quality. Pulpfree orange juice was processed at 20 kV/cm and 65°C with a hold time of 2 s. At these conditions, the population of *E. coli* was reduced by 3.9 log. Samples of orange juice were taken before and after RFEF processing and were analyzed for browning and ascorbic acid.

Vitamin C (ascorbic acid) was measured using a HPLC method as described earlier (*15*). Orange juice was centrifuged at 12,000 g for 10 min at 5°C in a Sorvall RC2-B refrigerated centrifuge (Kendro Laboratory Products, Newtown, CT). The supernatant was filtered through a 0.45 µm Acrodisc LC 13 PVDF syringe filter (Gelman Sciences, Ann Arbor, MI) before being analyzed using a Hewlett Packard Ti-series 1050 HPLC system (Agilent Technologies, Palo Alto, CA). The HPLC system consists of an autosampler, an integral photodiode-array detector, an autoinjector and a Hewlett-Packard Rev. A02.05 Chemstation. Injection volume was 20 µl. Separation of compounds was achieved with an Aminex HPX-87H organic acids column (300 × 7.8 mm) fitted with a microguard cation H+ eluted with a mobile phase of 5 mM sulfuric acid at flow-rate of 0.5 ml/min. Column temperature was maintained at 30°C using a column heater (Bio-Rad Laboratories, Hercules, CA). Ascorbic acid was monitored at 245 nm and calculated from an ascorbic acid standard.

To measure browning, orange juice was centrifuged at 12,000 g for 10 min at 5°C (*16*). The absorbance of the supernatant at 420 nm was measured using a spectrophotometer (Shimadzu UV-1601 spectrophotometer, Shimadzu Scientific Instruments, Columbia, MD).

Many fruit and fruit juices are rich in ascorbic acid (Vitamin C). Ascorbic acid is, however, sensitive to many processing and storage conditions. It is known that exposure to high temperatures during pasteurization results in a considerable loss of ascorbic acid. For example, pasteurization (90°C for 60 s) of fresh orange juice resulted in a 2.4% loss in ascorbic acid (*17*). No measurable loss in ascorbic acid was observed due to RFEF process (data not shown), probably due to the low treatment temperature and duration. The errors of analysis were probably larger than the loss (if any) of ascorbic acid. Uemura and Isobe (*18*) used a 20 kHz RFEF apparatus to study inactivation of *Bacillus subtilis* spores in orange juice. The orange juice was RFEF processed at 121°C under pressurized conditions to elevate the boiling point. A 16.3 kV/cm field reduced the viable *B. subtilis* spores by 4 log in <1 s of treatment. Only 10% of the original ascorbic acid in the orange juice was destroyed after RFEF treatment. In our experiment, the juice was RFEF processed at 65°C, a temperature much lower than 121°C.

Non-enzymatic browning is due to Maillard-type reactions of sugars, amino acids and ascorbic acid. The reactions, influenced by many factors (such as

temperature and oxygen), not only lead to browning and loss of ascorbic acid, but also produce compounds that contribute to off-flavor of juice. The oxidation of ascorbic acid can play an important role in the browning of fruit juice. No change in brownness of orange juice was observed as a result of RFEF treatment (data not shown), coinciding with the complete retention of ascorbic acid.

RFEF Nonthermal Inactivation of *E. coli* in Apple Cider

Escherichia coli K12 in apple cider was successfully inactivated at nonthermal conditions using the 80 kW RFEF pilot plant system. The extent of microbial inactivation is dependent on the electric field strength and temperature.

A series of experiments were performed at 21.1 kHz to determine the effects of electric field strength and temperature on inactivation. The RFEF process with three treatment chambers in series was used as shown in Figure 6. The treatment chambers used were the same as presented in Figure 5, except that the diameter and length of the central channel were 1.4 mm and 2.3 mm, respectively, and the space between the end of each of the electrodes and the central channel was 2.0 mm. The flow rate of cider was 1.5 l/min. The population of *E. coli* in apple cider was reduced by 2.4 log after being exposed to a 20 kV/cm peak electric field at a treatment time of 140 μs per treatment chamber, outlet temperature of 55°C, and hold time of 2 s per treatment chamber (Figure 11). Increasing the temperature to 60°C at the same field strength and time resulted in a reduction in *E. coli* of 5.0 log. When the cider was ohmicly heated at the same frequency, 21.1 kHz, to the same outlet temperature, 60°C, and held for the same time, 2 s per ohmic treatment chamber, the population of *E. coli* was reduced by only 0.2 log. Therefore, RFEF processing reduced the population of *E. coli* in apple cider by 4.8 log relative to the control. Previously, *E. coli* in apple juice was reduced by 2.1 log after being exposed to a 20 kV/cm peak electric field at a treatment time of 140 μs per treatment chamber, outlet temperature of 65°C, and hold time of 2 s using a RFEF pilot plant system with two treatment chambers in series such as shown in Figure 7 (*9*). The better results obtained with cider are probably due to the fact that 3 treatment chambers were employed rather than two, and that the total treatment time was 120% longer. The results of the present study successfully extended the RFEF process to inactivating *E. coli* in apple cider that contains solids.

Experiments were conducted to determine the effect of frequency on inactivation. The inactivation of *E. coli* in apple cider was similar as the frequency was varied from 21.1 to 40.1 kHz. In the case of *E. coli* in orange juice, presented earlier in this chapter, inactivation improved as the frequency decreased. The variation in results may be due to the use of different numbers of treatment chambers and treatment times. The effect of frequency needs to be studied in greater detail because, if greater inactivation occurs at lower frequencies, the energy operating costs could be reduced. In addition, RFEF equipment costs may be significantly less at lower frequencies.

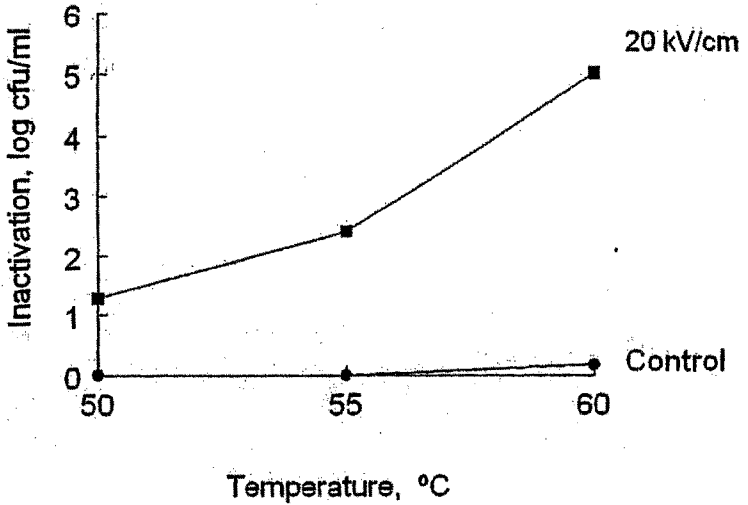

Figure 11. Effects of temperature and RFEF on the inactivation of E. coli at the following condition: 3 treatment chambers, 140 μs treatment time per treatment chamber, and 2 s hold time per treatment chamber (1.5 l/min flow rate). Means of two replicate experiments.

Scanning Electron Microscope Imaging of *E. coli* Inactivated Using RFEF

Very little is known about the mechanism of inactivation by either PEF or RFEF. In order to investigate this phenomenon, scanning electron microscope (SEM) images were produced for *E. coli* that were thermally treated, nonthermally treated using RFEF, and untreated.

Deionized water was inoculated with *E. coli* culture to yield an 8.6 log cfu/ml bacterial cell suspension. This high population was necessary to generate SEM images containing multiple bacteria. Water was used instead of apple cider because particulates had been found to interfere with the imaging of the bacteria. Hydrochloric acid was mixed with the water, before adding the cultures, to reduce the pH to 4.4 which is typical of cider. The populations of the untreated cells were not affected by the pH reduction.

For the RFEF treatments, the bacterial cell suspension was processed using the same setup as the apple cider, except that only two chambers were used instead of three as shown in Figure 6. The processing conditions were 25 kV/cm and 55°C with a hold time of 2 s per chamber. To increase the inactivation, the product was recycled back to the feed tank. In all, the bacterial cell suspension was processed 24 times for a total hold time at 55°C of 1.6 min. The population of *E. coli* was reduced by 4.8 log.

For the thermal treatments, 10 ml of the bacterial cell suspension was placed in a test tube and submerged in a water bath. To get an inactivation equivalent to that obtained using RFEF processing, the required time and temperature were 75°C and 5 min. The population of *E. coli* was reduced by 5.4 log. For comparison to the RFEF processing, a sample was held at 55°C for 5 min and the population of *E. coli* was reduced by less than 0.1 log.

Aliquots (50 μL) of bacterial cell suspensions were deposited onto 10 mm dia. glass coverslips. After ~ 30 s, the coverslips were gently immersed into 2 ml volumes of fixative solution, 2.5% glutaraldehyde-0.1M imidazole buffered at pH 7.0, in a multi-well plate. After 2 h at room temperature, the plate was sealed and stored at 40°C. In preparation for scanning electron microscopy, the fixative solution was removed and replaced with several ~2 ml aliquots of imidazole buffer to remove glutaraldehyde and bacterial cells on the coverslips were dehydrated by sequential immersion of the coverslips in aliquots of graded solutions of ethanol (50%, 80% and absolute) before critical point drying from liquid CO_2. The dried coverslips were glued to specimen stubs and coated with a thin layer of gold by DC sputtering in a ScanCoat 6 sputter coater (BOC Edwards, Wilminton, MA). Samples were viewed and digital images were collected using a Quanta 200 FEG scanning electron microscope (FEI Co., Inc., Hillsboro, OR) in the high vacuum, secondary electron imaging mode of operation.

Secondary electron images of the bacterial cells adhering to the glass coverslips after experimental and preparative treatments are illustrated in Figure 12. Control cells were typically individual, rod-shaped with smooth surfaces. But both groups of treated (thermal and nonthermal) cells were mostly loosely clumped into small groups containing three to ten cells with various superficial irregularities of the surface of nearly every cell. The shapes of thermally-treated cells were distorted by large, irregular depressions and evaginations of their surfaces. The shapes of nonthermally treated cells were less distorted than those thermal treated, but the visible surfaces had at least a few attached small vesicles.

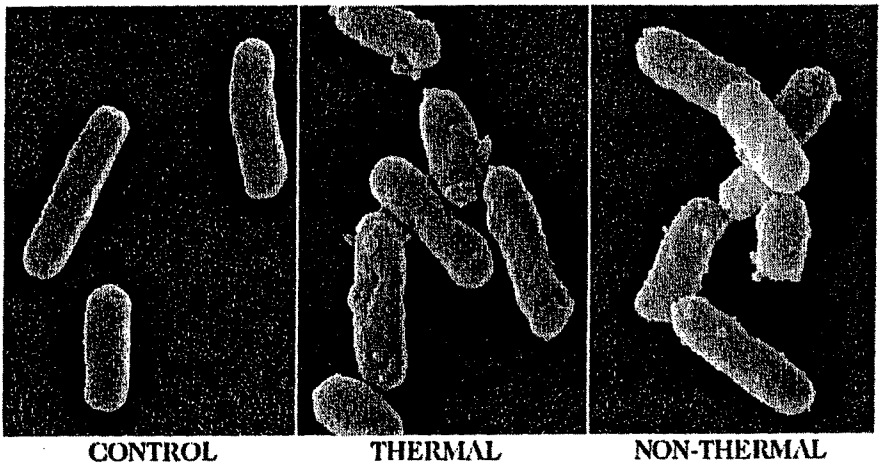

Figure 12. Scanning electron microscope images of untreated, thermally treated (at 75 °C for 5 min), and nonthermally treated E. coli using RFEF. The thermal and nonthermal inactivations were 5.4 and 4.8 log, respectively.

The radio frequency electric fields (RFEF) process has been shown to reduce the population of *Escherischia coli* in orange juice at 50°C. Inactivation is dependent upon the electric field strength and temperature. Better inactivation has been observed at radio frequencies near 20 kHz as compared to frequencies near 40 kHz. The calculated electrical cost is $0.0017 per liter of orange juice. There is no change in brownness of orange juice as a result of RFEF treatment, nor is there any loss of ascorbic acid. The RFEF process has also been shown to reduce the population of *E. coli* in apple cider at 50°C. Inactivation is dependent upon temperature, but, in this case, is independent of frequency. The difference in results may be due to the use of slightly different RFEF systems. Scanning electron microscope images of untreated, thermally treated, and RFEF

nonthermally treated *E. coli* showed noteworthy differences indicating that the mechanisms for thermal and RFEF inactivation are dissimilar.

Although remarkable progress has recently been made in the development of the nonthermal RFEF process, more research needs to be done before it can be commercialized. The RFEF process needs to be further scaled up to be of commercial interest. Additional quality and cost analyses must be performed. The stability of the equipment, including the metal electrodes, at longer operational times must be studied. Finally, RFEF processing at lower frequencies, where the efficiency may be enhanced, deserves attention.

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